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PCT/JP00/02710

PATENT COOPERATION TREATY

From the INTERNATIONAL BUREAU

PCT

NOTIFICATION OF ELECTION

(PCT Rule 61.2)

To:

Commissioner
US Department of Commerce
United States Patent and Trademark Office, PCT
2011 South Clark Place Room CP2/5C24
Arlington, VA 22202
ETATS-UNIS D'AMERIQUE

in its capacity as elected Office

Date of mailing (day/month/year) 29 November 2000 (29.11.00)	
International application No. PCT/JP00/02710	**Applicant's or agent's file reference** PWO-19659
International filing date (day/month/year) 25 April 2000 (25.04.00)	**Priority date** (day/month/year) 27 April 1999 (27.04.99)
Applicant TOJO, Takashi et al	

1. The designated Office is hereby notified of its election made:

[X] in the demand filed with the International Preliminary Examining Authority on:

11 October 2000 (11.10.00)

[ ] in a notice effecting later election filed with the International Bureau on:

2. The election [X] was

[ ] was not

made before the expiration of 19 months from the priority date or, where Rule 32 applies, within the time limit under Rule 32.2(b).

The International Bureau of WIPO **34, chemin des Colombettes** **1211 Geneva 20, Switzerland**	Authorized officer R. Forax
Facsimile No.: (41-22) 740.14.35	Telephone No.: (41-22) 338.83.38

Form PCT/IB/331 (July 1992)

JP0002710

PATENT COOPERATION TREATY

REC'D 13 JUL 2001
WIPO PCT

PCT

INTERNATIONAL PRELIMINARY EXAMINATION REPORT

(PCT Article 36 and Rule 70)

Applicant's or agent's file reference	FOR FURTHER ACTION	See Notification of Transmittal of International Preliminary Examination Report (Form PCT/IPEA/416)
PWO-19659		
International application No.	International filing date *(day/month/year)*	Priority date *(day/month/year)*
PCT/JP00/02710	25/04/2000	27/04/1999
International Patent Classification (IPC) or national classification and IPC C07K7/56		
Applicant FUJISAWA PHARMACEUTICAL CO., LTD.		

1. This international preliminary examination report has been prepared by this International Preliminary Examining Authority and is transmitted to the applicant according to Article 36.

2. This REPORT consists of a total of 7 sheets, including this cover sheet.

   ☐ This report is also accompanied by ANNEXES, i.e. sheets of the description, claims and/or drawings which have been amended and are the basis for this report and/or sheets containing rectifications made before this Authority (see Rule 70.16 and Section 607 of the Administrative Instructions under the PCT).

   These annexes consist of a total of sheets.

3. This report contains indications relating to the following items:

   - I ☒ Basis of the report
   - II ☐ Priority
   - III ☒ Non-establishment of opinion with regard to novelty, inventive step and industrial applicability
   - IV ☐ Lack of unity of invention
   - V ☒ Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations suporting such statement
   - VI ☐ Certain documents cited
   - VII ☐ Certain defects in the international application
   - VIII ☒ Certain observations on the international application

Date of submission of the demand	Date of completion of this report
11/10/2000	12.07.2001
Name and mailing address of the international preliminary examining authority: European Patent Office - P.B. 5818 Patentlaan 2 NL-2280 HV Rijswijk - Pays Bas Tel. +31 70 340 - 2040 Tx: 31 651 epo nl Fax: +31 70 340 - 3016	Authorized officer Groenendijk, M Telephone No. +31 70 340 3715

Form PCT/IPEA/409 (cover sheet) (January 1994)

INTERNATIONAL PRELIMINARY EXAMINATION REPORT

International application No. PCT/JP00/02710

I. Basis of the report

1. With regard to the **elements** of the international application *(Replacement sheets which have been furnished to the receiving Office in response to an invitation under Article 14 are referred to in this report as "originally filed" and are not annexed to this report since they do not contain amendments (Rules 70.16 and 70.17))*:
Description, pages:

424 as originally filed

Claims, No.:

1-12 as originally filed

2. With regard to the **language**, all the elements marked above were available or furnished to this Authority in the language in which the international application was filed, unless otherwise indicated under this item.

These elements were available or furnished to this Authority in the following language: , which is:

- ☐ the language of a translation furnished for the purposes of the international search (under Rule 23.1(b)).
- ☐ the language of publication of the international application (under Rule 48.3(b)).
- ☐ the language of a translation furnished for the purposes of international preliminary examination (under Rule 55.2 and/or 55.3).

3. With regard to any **nucleotide and/or amino acid sequence** disclosed in the international application, the international preliminary examination was carried out on the basis of the sequence listing:

- ☐ contained in the international application in written form.
- ☐ filed together with the international application in computer readable form.
- ☐ furnished subsequently to this Authority in written form.
- ☐ furnished subsequently to this Authority in computer readable form.
- ☐ The statement that the subsequently furnished written sequence listing does not go beyond the disclosure in the international application as filed has been furnished.
- ☐ The statement that the information recorded in computer readable form is identical to the written sequence listing has been furnished.

4. The amendments have resulted in the cancellation of:

- ☐ the description, pages:
- ☐ the claims, Nos.:
- ☐ the drawings, sheets:

5. ☐ This report has been established as if (some of) the amendments had not been made, since they have been considered to go beyond the disclosure as filed (Rule 70.2(c)):

(Any replacement sheet containing such amendments must be referred to under item 1 and annexed to this report.)

6. Additional observations, if necessary:

III. Non-establishment of opinion with regard to novelty, inventive step and industrial applicability

1. The questions whether the claimed invention appears to be novel, to involve an inventive step (to be non-obvious), or to be industrially applicable have not been examined in respect of:

☐ the entire international application.

☒ claims Nos. 12 as to industrial applicability.

because:

☒ the said international application, or the said claims Nos. 12 as to industrial applicability relate to the following subject matter which does not require an international preliminary examination (*specify*):
see separate sheet

☐ the description, claims or drawings (*indicate particular elements below*) or said claims Nos. are so unclear that no meaningful opinion could be formed (*specify*):

☐ the claims, or said claims Nos. are so inadequately supported by the description that no meaningful opinion could be formed.

☐ no international search report has been established for the said claims Nos. .

2. A meaningful international preliminary examination cannot be carried out due to the failure of the nucleotide and/or amino acid sequence listing to comply with the standard provided for in Annex C of the Administrative Instructions:

☐ the written form has not been furnished or does not comply with the standard.

☐ the computer readable form has not been furnished or does not comply with the standard.

V. Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement

1. Statement

Novelty (N)	Yes:	Claims	1-12
	No:	Claims	
Inventive step (IS)	Yes:	Claims	
	No:	Claims	1-12
Industrial applicability (IA)	Yes:	Claims	1-11

No: Claims

2. Citations and explanations
see separate sheet

VIII. Certain observations on the international application

The following observations on the clarity of the claims, description, and drawings or on the question whether the claims are fully supported by the description, are made:
see separate sheet

Re Item III

Non-establishment of opinion with regard to novelty, inventive step and industrial applicability

Claims 12 relates to subject-matter considered by this Authority to be covered by the provisions of Rule 67.1(iv) PCT. Consequently, no opinion will be formulated with respect to the industrial applicability of the subject-matter of these claims (Article 34(4)(a)(i) PCT).

Re Item V

Reasoned statement under Rule 66.2(a)(ii) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement

Reference is made to the following documents:

D1:EP-A-0644199
D2:EP-A-0535959
D3Bioorg.Med.Chem.Lett., Vol.5, NO.20, 1995, 2357-2362
D4:WO-A-9611210

I.NOVELTY

In view of the available prior art the claims 1-12 are considered to be novel under Art.33(2) PCT.

II.INVENTIVE STEP

1)The closest prior art is considered to be D1, disclosing cyclic hexapeptides of the present type having antimicrobial activity.
2)The compounds of the application essentially only differ from this prior art in the presence of a (substituted) [SPEC0803]-hydroxy-ornithine residue as substitute for the (substituted) [SPEC0803]-hydroxy-glutamine residue. These compounds appear to exhibit an activity similar to the prior art compounds.
3)The problem to be solved may therefore be considered to be the provision of alternative cyclohexapeptides having antimicrobial activity.
4)However D2 and D3, which documents relate to the same type of compounds with

the same activity as the compounds of the application and D1, already disclosed the substitution of the [SPEC0803]-hydroxy-glutamine residue by a [SPEC0803]-hydroxy-ornithine residue. Having regard to the table on page 3 of D2 and the comparative data in the tables 1 and 2 of D3 it is considered that there is a relatively large freedom in the substitution pattern without a detrimental effect on the activity.
The examiner is therefore of the opinion that the introduction of the [SPEC0803]-hydroxy-ornithine residue in the compounds of D1 is merely based on a selection out of several possibilities from which a skilled person would select, without the exercise of inventive skill, in order to solve the problem posed.
5)Furthermore the present claims allow a plethora of substituents on the amino group of the [SPEC0803]-hydroxy-ornithine residue and as sidechain, N-terminally connected to the [SPEC0807]- hydroxy-ornithine residue.
However many of said substituents have already been disclosed in D1 and, as regards the N-terminally connected sidechain, D4 (e.g., see examples 1-124). Their application in the present compounds is therefore also considered to be within the normal skill of an artisan.
6)Therefore in order to acknowledge an inventive step to the present compounds, they should exhibit unexpected advantageous properties compared to the prior art compounds of D1. However said properties have neither been posed by the application nor have they become plausible otherwise.
5)Consequently the claims 1-12 are considered to lack an inventive step under Art.33(3) PCT.

For the assessment of the present claims 10-12 on the question whether they are industrially applicable, no unified criteria exist in the PCT Contracting States. The patentability can also be dependent upon the formulation of the claims. The EPO, for example, does not recognize as industrially applicable the subject-matter of claims to the use of a compound in medical treatment, but may allow, however, claims to a known compound for first use in medical treatment and the use of such a compound for the manufacture of a medicament for a new medical treatment.

Re Item VIII

Certain observations on the international application

1)An independent claim should clearly specify all of the essential features needed to define the invention PCT Guidelines C-III,4.1-4.7a). The present claims 1,2 and 5 contain expressions like "lower alkyl", "acyl group""heterocyclic group" all facultatively containing one or more "suitable substituent(s)", rendering the scope of said claims unclear under Art.6 PCT. It is true that under circumstances such expressions can be acceptable in product claims, e.g. in definitions of non-essential features like protecting groups. However in the present case said expressions are also used to define structural features that are considered to be characteristic for the present compounds. Consequently the claims 1,2 and 5 are considered not to fulfil the requirements of Art.6 PCT.

2)The examples 22,41,43,44,87,99,100 and 126 are not encompassed by the claims.

PATENT COOPERATION TREATY

From the INTERNATIONAL BUREAU

PCT

NOTICE INFORMING THE APPLICANT OF THE COMMUNICATION OF THE INTERNATIONAL APPLICATION TO THE DESIGNATED OFFICES

(PCT Rule 47.1(c), first sentence)

To:
TABUSHI, Eiji
Fujisawa Pharmaceutical Co., Ltd.
Osaka Factory
1-6, Kashima 2-chome
Yodogawa-ku, Osaka-shi
Osaka 532-8514
JAPON

Date of mailing (day/month/year) 02 November 2000 (02.11.00)		
Applicant's or agent's file reference PWO-19659	**IMPORTANT NOTICE**	
International application No. PCT/JP00/02710	**International filing date** (day/month/year) 25 April 2000 (25.04.00)	**Priority date** (day/month/year) 27 April 1999 (27.04.99)
Applicant FUJISAWA PHARMACEUTICAL CO., LTD. et al		

1. Notice is hereby given that the International Bureau has communicated, as provided in Article 20, the international application to the following designated Offices on the date indicated above as the date of mailing of this Notice:
   US

   In accordance with Rule 47.1(c), third sentence, those Offices will accept the present Notice as conclusive evidence that the communication of the international application has duly taken place on the date of mailing indicated above and no copy of the international application is required to be furnished by the applicant to the designated Office(s).

2. The following designated Offices have waived the requirement for such a communication at this time:
   EP,JP

   The communication will be made to those Offices only upon their request. Furthermore, those Offices do not require the applicant to furnish a copy of the international application (Rule 49.1(a-bis)).

3. Enclosed with this Notice is a copy of the international application as published by the International Bureau on
   02 November 2000 (02.11.00) under No. WO 00/64927

REMINDER REGARDING CHAPTER II (Article 31(2)(a) and Rule 54.2)

If the applicant wishes to postpone entry into the national phase until 30 months (or later in some Offices) from the priority date, **a demand for international preliminary examination** must be filed with the competent International Preliminary Examining Authority before the expiration of 19 months from the priority date.

It is the applicant's sole responsibility to monitor the 19-month time limit.

Note that only an applicant who is a national or resident of a PCT Contracting State which is bound by Chapter II has the right to file a demand for international preliminary examination.

REMINDER REGARDING ENTRY INTO THE NATIONAL PHASE (Article 22 or 39(1))

If the applicant wishes to proceed with the international application in the **national phase**, he must, within 20 months or 30 months, or later in some Offices, perform the acts referred to therein before each designated or elected Office.

For further important information on the time limits and acts to be performed for entering the national phase, see the Annex to Form PCT/IB/301 (Notification of Receipt of Record Copy) and Volume II of the PCT Applicant's Guide.

The International Bureau of WIPO **34, chemin des Colombettes** **1211 Geneva 20, Switzerland**	Authorized officer J. Zahra
Facsimile No. (41-22) 740.14.35	Telephone No. (41-22) 338.83.38

Form PCT/IB/308 (July 1996)

PATENT COOPERATION TREATY

From the INTERNATIONAL BUREAU

PCT

NOTIFICATION CONCERNING SUBMISSION OR TRANSMITTAL OF PRIORITY DOCUMENT

(PCT Administrative Instructions, Section 411)

To:

TABUSHI, Eiji
Fujisawa Pharmaceutical Co., Ltd.
Osaka Factory
1-6, Kashima 2-chome
Yodogawa-ku, Osaka-shi
Osaka 532-8514
JAPON

Date of mailing (day/month/year) 08 June 2000 (08.06.00)	
Applicant's or agent's file reference PWO-19659	**IMPORTANT NOTIFICATION**
International application No. PCT/JP00/02710	International filing date (day/month/year) 25 April 2000 (25.04.00)
International publication date (day/month/year) Not yet published	Priority date (day/month/year) 27 April 1999 (27.04.99)
Applicant FUJISAWA PHARMACEUTICAL CO., LTD. et al	

1. The applicant is hereby notified of the date of receipt (except where the letters "NR" appear in the right-hand column) by the International Bureau of the priority document(s) relating to the earlier application(s) indicated below. Unless otherwise indicated by an asterisk appearing next to a date of receipt, or by the letters "NR", in the right-hand column, the priority document concerned was submitted or transmitted to the International Bureau in compliance with Rule 17.1(a) or (b).

2. This updates and replaces any previously issued notification concerning submission or transmittal of priority documents.

3. An **asterisk(*)** appearing next to a date of receipt, in the right-hand column, denotes a priority document submitted or transmitted to the International Bureau but not in compliance with Rule 17.1(a) or (b). In such a case, **the attention of the applicant is directed** to Rule 17.1(c) which provides that no designated Office may disregard the priority claim concerned before giving the applicant an opportunity, upon entry into the national phase, to furnish the priority document within a time limit which is reasonable under the circumstances.

4. The **letters "NR"** appearing in the right-hand column denote a priority document which was not received by the International Bureau or which the applicant did not request the receiving Office to prepare and transmit to the International Bureau, as provided by Rule 17.1(a) or (b), respectively. In such a case, **the attention of the applicant is directed** to Rule 17.1(c) which provides that no designated Office may disregard the priority claim concerned before giving the applicant an opportunity, upon entry into the national phase, to furnish the priority document within a time limit which is reasonable under the circumstances.

Priority date	Priority application No.	Country or regional Office or PCT receiving Office	Date of receipt of priority document
27 Apri 1999 (27.04.99)	PP9997	AU	26 May 2000 (26.05.00)

The International Bureau of WIPO
34, chemin des Colombettes
1211 Geneva 20, Switzerland

Facsimile No. (41-22) 740.14.35

Authorized officer

Somsak Thiphrakesone

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Bioorganic & Medicinal Chemistry Letters, Vol. 5, No. 20, pp. 2357-2362, 1995

0960-894X/95 $9.50+0.00

0960-894X(95)00407-6

ANTIFUNGAL LIPOPEPTIDES: STRUCTURE-ACTIVITY RELATIONSHIPS OF 3-HYDROXYGLUTAMINE-MODIFIED PNEUMOCANDIN B_0 DERIVATIVES

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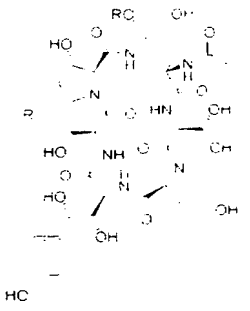
Departments of [a]Medicinal Chemistry, [b]Infectious Disease Research, [c]Parasite Biochemistry and Cell Biology, and [d]Antibiotic Discovery and Development
Merck Research Laboratories, P.O. Box 2000, Rahway, New Jersey 07065-0900

Abstract: Selective methanolysis or dehydration followed by reduction of the 3-hydroxyglutamine residue of pneumocandin B_0 (**1**) or its dideoxy analog **5** (L-692,289) gave the methyl 3-hydroxyglutamate and 3-hydroxyornithine analogs **6** and **9**, respectively. Further derivatization of these analogs allowed a study of the SAR at this position. In general, carboxylic acid-containing derivatives were poorer antifungal agents than neutral derivatives while amine-bearing analogs displayed the greatest potency.

Introduction

The incidence of serious fungal infection has steadily grown over the last two decades despite the introduction of a number of new agents. Immunosuppression from AIDS, anticancer therapy, the use of broad spectrum antibiotics and chemotherapy in organ transplantation accounts for this growing trend.[1] The majority of life-threatening fungal infections are caused by opportunistic pathogens such as *Candida* spp., *Aspergillus* spp., *Pneumocystis carinii* and *Cryptococcus neoformans*.[2] Currently available antifungal agents suffer drawbacks due to toxicity, static rather than cidal activity or inadequate spectrum. In addition, in some cases the selection of resistant organisms has been seen as the usage of these agents has increased.[3] Therefore, there is a considerable need for the development of new antifungal agents with improved properties.



		OR	R'
1	Pneumocandin B_0	OH	$CONH_2$
2	L-705,589	$O(CH_2)_2NH_2$	$CONH_2$
3	L-731,373	OH	CH_2NH_2
4	L-733,560	$O(CH_2)_2NH_2$	CH_2NH_2

The pneumocandins belong to a class of closely related fungicidal lipopeptides isolated from the fungus *Zalerion arboricola*.[4] Like the structurally-related echinocandins, these compounds inhibit the synthesis of β-1,3-glucan, an essential component of the fungal cell wall that is absent in mammalian cells. Thus, the inhibition of β-1,3-glucan synthesis represents a fungal-specific, potentially non-toxic target. Pneumocandin B_0 **(1)**, a cyclic hexapeptide possessing a 10,12-dimethylmyristoyl side chain, has provided an important platform for the synthesis of potent fungicidal derivatives. Recently, Bouffard, *et al.* have described several cationic derivatives of **1**.[5] L-705,589 **(2)**, L-731,373 **(3)**, and L-733,560 **(4)** are potent inhibitors of β-1,3-glucan synthase with excellent *in vitro* activity and efficacy in rodent models of disseminated candidiasis, aspergillosis and *P. carinii* pneumonia.[6] Compounds **3** and **4** possess a modified 3-hydroxyglutamine residue (gln→orn). In this report, we wish to expand on the structure-activity relationships at the 3-hydroxyglutamine (3-OH *gln*) position.

Biological Assays

The β-1,3-glucan synthase inhibition assay was conducted using a crude membrane system derived from *C. albicans* (MY 1208) as previously described.[7] An IC_{50} (μM) was determined and refers to the concentration of drug required to inhibit the production of 50% of the insoluble glucan compared to the control.

Fungicidal activity was determined against a panel of *Candida* spp., and *Cryptococcus neoformans* (in duplicate).[6a] The MFC or minimum fungicidal concentration is defined as the concentration of drug (μg/mL) that inhibits regrowth of the organism. Compounds showed weak to no activity (32 - >128 μg/mL) against *C. neoformans*. Data are presented for *C. albicans* and the inherently more resistant *C. parapsilosis*.

The *in vivo* anti-*Candida* activity was determined in a mouse model of disseminated candidiasis (TOKA).[8] Mice (n=5) were infected I.V. with a 50% lethal dose of *C. albicans* (MY 1055) and dosed I.P. BID for 4 days with drug. On day 7 post-infection, the kidney burden was quantitated and an effective dose (mg/kg/dose) for at least 99.9% reduction in colony forming units (CFUs) as compared to control animals was determined ($ED_{99.9}$).

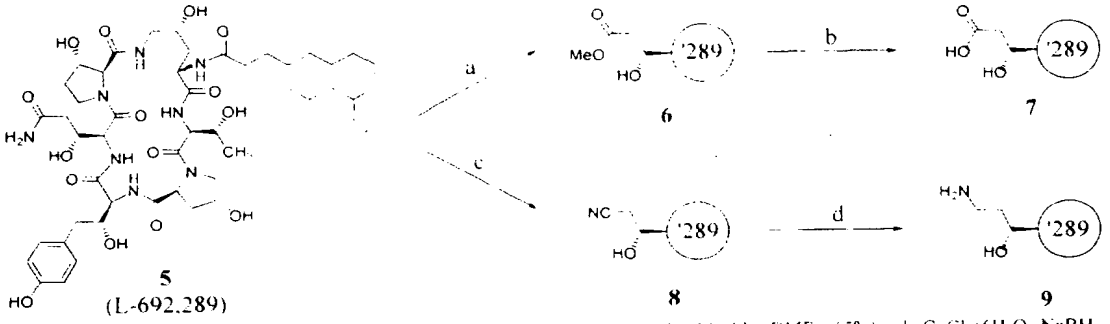
Chemistry

The 3-OH *gln* residue was envisioned to undergo selective hydrolysis to a 3-OH *glu* or selective reduction to a 3-OH *orn*. Since **1** is unstable at low and high pH,[9] we first investigated the chemistry of the stable dideoxy-analog, L-692,289 **(5)**.[10] Selective hydrolysis was accomplished by acid-catalyzed methanolysis to give **6**[11] followed by basic hydrolysis of the methyl ester to give **7**. The selective dehydration of the primary amide of **5** afforded nitrile **8** which was reduced to the 3-OH *orn* analog **9** using in situ-generated cobalt boride

and sodium borohydride in methanol[12] (Scheme 1). With these key intermediates available, the preparation of compounds **10-16** could be accomplished (see Table 1).

The hydroxamic acid **10** and hydrazide **11** were prepared by treatment of ester **6** with either hydroxylamine hydrochloride and aqueous sodium hydroxide in methanol or hydrazine in methanol in 35% and 78% yields, respectively. Carboxylic acid **7** was obtained as a by-product in the formation of **10** in 20% yield. The reduction of ester **6** to the carbinol **12** was accomplished with 4 molar equivalents of $LiBH_4$ in isopropanol in 20% yield. The relatively lipophilic thioamide **13** was obtained from nitrile **8** by treatment with hydrogen sulfide gas in a mixture of diethylamine/DMF (1:3) at 60 °C in 35% yield. Amides **14** and **15** were prepared from acid **7** and the corresponding amine employing 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride and 1-hydroxybenzotriazole in DMF in 44% and 69% yields, respectively. Hydrolysis of methyl ester **15** gave the carboxylic acid **16**. The cationic products were isolated as their TFA salts.

Scheme 1. Selective Hydrolysis or Reduction of the 3-Hydroxyglutamine Residue of **5**



(a) CSA, MeOH, 45 °C (20%); (b) 2N NaOH (aq), MeOH (66%); (c) cyanuric chloride, DMF, (65%); (d) $CoCl_2 \bullet 6H_2O$, $NaBH_4$, MeOH (50%)

Attempted methanolysis of **1** was unsuccessful leading to solvolysis of the C-5 ornithine and C-4 homotyrosine hydroxyl groups. The selective dehydration of the glutamine residue could be accomplished to give nitrile **17** (see Table 2) by carefully controlling the cyanuric chloride stoichiometry, reaction time and temperature as previously described.[5] The crude product was reduced with cobalt (II) chloride and sodium borohydride in methanol to give an overall 44% yield of the primary amine **3**. Compound **3** was acylated with acetic anhydride and diisopropylethylamine in DMF to give **18** in 85% yield. Alkylation of **3** with excess bromoacetonitrile gave the dialkylated adduct **19** in 44% yield with none of the quaternary analog detected. Synthesis of the methylamino analog **20** first required reductive alkylation to the N-benzyl adduct (Structure B, R = $-CH_2NHCH_2C_6H_5$) using benzaldehyde and sodium cyanoborohydride in DMF containing 1% acetic acid (49% yield). Next, methylation with 37% formaldehyde and sodium cyanoborohydride in aqueous acetonitrile gave the N-methyl-N-benzyl adduct in 72% yield. Hydrogenolysis of the benzyl group under 1 atm of H_2 with

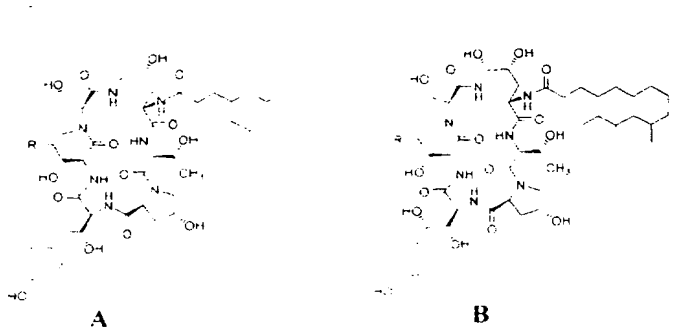
10% Pd-C as catalyst gave **20** in 84% yield. The N,N-dimethyl adduct **21** was obtained by treatment of **3** with 37% formaldehyde and sodium cyanoborohydride in aqueous acetonitrile. The quaternary ammonium analog **22** was obtained by treatment of **21** with excess MeI in DMF. The guanidine analog **23** was prepared from **3** by treatment with formamidinesulfonic acid[13] in the presence of Hunig's base in 46% yield. Satisfactory 400 MHz ^{1}H-NMR spectra (CD_3OD) and FAB-MS were obtained for all compounds. Final compounds were purified by preparative reverse phase HPLC (C8 or C18 ZORBAX, acetonitrile-water-0.1% TFA) and were >92% pure by analytical HPLC (λ=210 nm).

Results

The *in vitro* and *in vivo* anti-*Candida* activities of pneumocandin B_0 (**1**) and its dideoxy-analog **5** are quite similar[7] allowing a valid comparison between derivatives of either of these compounds. Indeed, nitrile analogs **8** and **17** and amine analogs **9** and **3** also display similar activities (see Tables 1 and 2). Thus, the SAR from series A can be assumed to parallel that from series B.

The β-1,3-glucan synthase enzyme assay is a crude membrane preparation where the cell wall has been digested and the disrupted plasma membrane and its components have been separated by centrifugation. Thus, it is not a pure enzyme and contains lipids and other materials that may influence the "activity" of a compound based on the compound's physicochemical properties. With this in mind, several general structure-activity relationships were apparent from the enzyme inhibition data. Neutral groups at the 3-OH *gln* position, whether polar (**1**, **5**, **10**, **11** and **12**) or lipophilic (**6**, **8**, **13**, **15** and **17**), possessed similar activity. Compounds possessing a carboxylic acid substituent (**7** and **16**) were poorer inhibitors than the neutral analogs. With the amine analogs, a substantial increase in potency was noted that roughly correlated with the basicity of the amine. The basic analogs **3**, **9**, **14**, **20**, **21**, **22** and **23** had significantly lower IC_{50}s than **1** or **5** but the non-basic amine analog **19** was substantially less active especially when compared to **21**. Alkyl substitution of the amine had little influence on enzyme activity (**3**, **20**, **21** and **22**). The acetamide derivative **18** was a fourfold poorer inhibitor than **1** suggesting that a carbonyl group is unfavorable in this position. Nonetheless, the isosteric and basic guanidine analog **23** showed a tenfold increase in activity relative to **1** and at least a 28-fold increase compared to **18**, highlighting the positive influence of a basic substituent at that position.

The *in vitro* fungicidal activity (MFC) of the compounds against two different *Candida* species is shown in Tables 1 and 2. The *C. albicans* (MY 1055) is a clinical isolate and is the organism used in the *in vivo* TOKA model. The *C. parapsilosis* (MY 1010) is a species that is inherently more resistant to the lipopeptides. Although the MFC's did not correlate completely with glucan synthase inhibition, several of the amine analogs (**3**, **22** and **23**) displayed potent activity against both *Candida* species. The monomethyl and dimethylamino analogs **20** and **21** were less potent against the whole organism even though they were potent enzyme inhibitors.



A B

Table 1. Biological Data for Dideoxy-Pneumocandin B_0 Analogs (Structure A)

(Structure A) R		Glucan Synthase IC_{50} (μM)	*In Vitro* MFC (μg/mL) *C. albicans* (MY 1055)	*In Vitro* MFC (μg/mL) *C. parap.* (MY 1010)	*In Vivo* TOKA $ED_{99.9}$ (mg/kg)
(5)	$-CONH_2$	0.07	0.25	2	>6 (2.93)[a]
(6)	$-CO_2Me$	0.18	0.5	4	>6 (0)[a]
(7)	$-CO_2H$	0.4	0.25	8	>6 (0)[a]
(8)	$-CN$	0.1	1	4	---
(9)	$-CH_2NH_2$	0.01	0.125	---	1.5
(10)	$-CONHOH$	0.08	0.25	4	6
(11)	$-CONHNH_2$	0.11	4	8	---
(12)	$-CH_2OH$	0.2	1	8	---
(13)	$-CSNH_2$	0.12	0.25	4	>6 (0)[a]
(14)	$-CONH(CH_2)_6NH_2$	0.038	0.5	---	>6 (1.6)[a]
(15)	$-CONH(CH_2)_5CO_2Me$	0.25	4	>128	---
(16)	$-CONH(CH_2)_5CO_2H$	0.9	2	64	---

[a]log reduction in CFUs at indicated dose

Table 2. Biological Data for Pneumocandin B_0 Analogs (Structure B)

(Structure B) R		Glucan Synthase IC_{50} (μM)	*In Vitro* MFC (μg/mL) *C. albicans* (MY 1055)	*In Vitro* MFC (μg/mL) *C. parap.* (MY 1010)	*In Vivo* TOKA $ED_{99.9}$ (mg/kg)
(1)	$-CONH_2$	0.07	0.25	1	6
(3)	$-CH_2NH_2$	0.01	<0.06	0.5	0.375
(17)	$-CN$	0.1	2	2	---
(18)	$-CH_2NHAc$	0.3	4	8	12
(19)	$-CH_2N(CH_2CN)_2$	0.2	4	8	>1.5 (0.94)[a]
(20)	$-CH_2NHMe$	0.007	2	2	0.375
(21)	$-CH_2NMe_2$	0.005	1	2	1.5
(22)	$-CH_2NMe_3^+$	0.009	0.125	0.5	0.375
(23)	$-CH_2NHC(=NH)NH_2$	0.007	<0.06	0.5	1.5

[a]log reduction in CFUs at indicated dose

The *in vivo* activity correlated well with the glucan synthase assay. The 3-OH *orn* analog of pneumocandin B_0 **3** was fourfold more potent than the corresponding dideoxy-analog **9**. Compound **3** and its trimethylammonium derivative **22** were the most potent compounds tested. Similar to the MFC assay, the monomethyl and dimethyl analogs were approximately two- to fourfold less potent.

In summary, cationic substituents at the 3-OH *gln* position of the pneumocandins significantly increased the enzyme, whole cell activity and *in vivo* potency of this class of compounds. Anionic groups, such as carboxylate, decreased the activity of analogs while neutral groups generally had little effect on activity.

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11. Preparation of **6**. p-Toluenesulfonic acid monohydrate (0.25 g, 1.3 mmol) was added to a solution of **5** (1.0 g, 0.97 mmol) in 40 mLs of methanol. The reaction vessel was heated to 49 °C and sealed. After stirring at 45-49 °C for 120 h, HPLC analysis showed a ratio of 1.6:1 for **6**:**5**. The mixture was concentrated *in vacuo* and purified by reverse phase HPLC (22.5 x 500 mm C8 ZORBAX, 57% acetonitrile in water). The appropriate fractions were lyophilized to give 200 mg (20%) of **6** as a white powder of 97% purity (λ=210 nm).
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